UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/620,777	07/15/2003	Roy Curtiss III	56029/	1127

7590 10/31/2007

Leon R. Yankwich, Esq.
YANKWICH & ASSOCIATES
201 Broadway
Cambridge, MA 02139

EXAMINER	
GANGLE, BRIAN J	
ART UNIT	PAPER NUMBER
1645	

MAIL DATE	DELIVERY MODE
10/31/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/620,777	CURTISS ET AL.	
	Examiner	Art Unit	
	Brian J. Gangle	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 August 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *61-75,77-86 and 88-103* is/are pending in the application.

4a) Of the above claim(s) *65,66,68,69,74,75,77-82 and 95-103* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *61-64, 67, 70-73, 83-86, and 88-94* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's remarks and amendment, filed 8/20/2007, are acknowledged. Claims 61, 63, 83, 84, 85, 88, and 89 are amended. Claims 76, 87, and 104-107 are cancelled. Claims 61-75, 77-86, and 88-103 are pending. Claims 65-66, 68-69, 74-75, 77-82, and 95-103 are withdrawn as being drawn to non-elected inventions. Claims 61-64, 67, 70-73, 83-86, and 88-94 are currently under examination.

Election/Restrictions

Applicant continues to traverse the previous restriction requirement. Applicant is reminded that the restriction requirement has been made final and that applicant is free to file a petition regarding the restriction requirement. It is noted that applicant asserts that the application shows that the essential genes are interchangeable in the ELVS system. If applicant intends this statement to mean that the various essential genes are obvious variants, they should clearly state that the essential genes are obvious variants of one another, and the restriction with regard to these essential genes will be withdrawn.

The restriction requirement is maintained and will not be revisited.

Claim Objections Maintained

The objection to claims 61-64, 67, 70-72, 83-86, 88-90, and 92-94, because the claims are drawn, in part, to non-elected subject matter, is maintained. While the claims have been amended to include *asd* as the essential gene, the claims have not been amended to restrict the invention to where the essential gene is located extrachromosomally.

New Claim Objections

Claims 88 and 91-94 are objected to because of the following informalities:

Claim 88 is substantially duplicative of claim 61.

Claim 92 is substantially duplicative of claim 70.

Claim 93 is substantially duplicative of claim 71.

Claim 94 is substantially duplicative of claim 72.

Claim 91 is substantially duplicative of claim 73.

Appropriate correction is required.

Claim Rejections Withdrawn

The rejection of claims 61-62, 70-71, 73, 84, 88, 91-94, and 104-107, on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 6-9 of U.S. Patent No. 5,840,483, is withdrawn in light of applicant's amendment thereto.

The rejection of claims 61-62, 70-73, 84, 88, 91-94, and 104-107, on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, and 7-8 of U.S. Patent No. 5,672,345, is withdrawn in light of applicant's amendment thereto.

The rejection of claims 61-64, 67, 73, 83-89, and 104-107, under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement, is withdrawn in light of applicant's amendment thereto.

The rejection of claims 61-64, 67, 73, 83-89, and 104-107, under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for bacterial cells containing the claimed environmentally limited viability system, does not reasonably provide enablement for microbial cells containing the claimed environmentally limited viability system, is withdrawn in light of applicant's amendment thereto.

The rejection of claims 61, 84, and 87, under 35 U.S.C. 112, second paragraph, as being rendered vague and indefinite by the use of the word "temporarily," is withdrawn in light of applicant's amendment thereto.

The rejection of claims 85 and 89 under 35 U.S.C. 112, second paragraph, as being rendered vague and indefinite because the term "about" in claims is a relative term, is withdrawn in light of applicant's amendment thereto.

The rejection of claims 61-62, 70-73, 83-84, 87-88, 91-94, and 104-107 under 35 U.S.C. 102(b) as being anticipated by Galan *et al.* (Gene, 94:29-35, 1990), is withdrawn in light of applicant's amendment thereto.

The rejection of claims 61-62, 70-73, 84, 88, 91-94, and 104-107 under 35 U.S.C. 102(e) as being anticipated by Curtiss (US Patent 5,840,483), is withdrawn in light of applicant's amendment thereto.

The rejection of claims 61-62, 70-73, 84, 88, 91-94, and 104-107 under 35 U.S.C. 102(e) as being anticipated by Curtiss (US Patent 5,672,345), is withdrawn in light of applicant's amendment thereto.

Claim Rejections Maintained

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

The rejection of claims 61-64, 67, 70-73, and 83-94 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is maintained for the reasons set forth in the previous office action.

Claims 61, 84, and 87-88 are rendered vague and indefinite by the use of the term "viable."

Applicant argues:

1. That the term "viable" would be fully understood by persons skilled in the art.

2. That a person skilled in the art would "fully appreciate that the cells would be viable, that is, possess the essential genes and characteristics for cell survival and propagation, in the permissive environment and would be non-viable, that is, lack essential genes or proteins necessary for cell survival and propagation, in the non-permissive environment."

Applicant's arguments have been fully considered and deemed non-persuasive.

Regarding argument 1, those of skill in the art know that there has been an extensive and ongoing debate for many years over what constitutes a "viable" cell (see for example, Bogosian *et al.*, EMBO Reports, 21:770-774, 2001; Kell *et al.*, Antonie van Leeuwenhoek, 73:169-187, 1998; Oliver, J. Microbiol., 43:93-100, 2005). Some equate "viability" with "culturability," while others accept that "viable but non-culturable" cells are alive and viable, but cannot be grown in culture. In the instant case, applicant has not stated whether a "viable" cell is one which is currently growing, or one which is capable of growing. Clearly, there is ambiguity about what this term means.

Regarding argument 2, applicant's remarks are at odds with both the specification and the claims. The specification defines a "non-viable" organism as one that cannot grow. According to applicant's remarks, a non-viable cell "lacks essential genes or proteins necessary for cell survival and propagation, in the non-permissive environment." First, cells do not lose and gain genes as they pass from a permissive environment to a non-permissive environment. They would have the same genes, but would change the level of expression of those genes. Second, the claims require that the cell have the essential gene. If it did not, it would not meet the limitations of the claim. Finally, applicant's comments regarding cell survival and propagation illustrate the ambiguity of the terms "viable" and "non-viable." Growth and propagation are not equivalent. Cells can increase in size or can have metabolic activity without propagating.

As outlined previously, the specification defines a "non-viable" cell as one that cannot grow. Does this mean that the definition of a "viable" cell is one that can grow? If so, does applicant intend a viable cell to be one which is currently growing, or one which is capable of growing? If the latter is the case, the only way for a cell to be non-viable is to be dead.

35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

The rejection of claims 61-63, 67, 70-73, 83-84, 88, and 91-94, under 35 U.S.C. 103(a) as being unpatentable over Galan *et al.* (Gene, 94:29-35, 1990) in view of Guzman *et al.* (J. Bacteriol., 177:4121-4130, 1995), is maintained for the reasons set forth in the previous office action.

Applicant argues:

1. That Galan does not teach or suggest regulating the expression of *asd*. Instead, Galan teaches the use of "balanced-lethal" technology, where the *asd* gene is deleted from the bacterial chromosome and is supplied on a plasmid, to serve as a selection pressure to maintain the plasmid.

2. That there is no teaching or suggestion in Galan or Guzman to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another. Applicant asserts that this system is useful for vaccine microorganisms.

Applicant's arguments have been fully considered and deemed non-persuasive.

Regarding argument 1, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Regarding argument 2, the motivation to combine was set forth in the previous office action. As stated previously, Galan teaches a bacterial strain where the *asd* gene has been deleted from the bacterial chromosome and is supplied on a plasmid. Guzman teaches a promoter system that would allow expression only in the presence of arabinose (the same promoter system used by applicant in claim 67). Guzman further teaches that it is useful to link

their promoter system to essential genes to study the depletion phenotype created by a null mutation. Therefore, one would have been motivated to link *asd* to the promoter of Guzman to study depletion phenotypes. Further, according to *KSR International Co. v. Teleflex Inc.*, No. 04-1350 (U.S. Apr. 30, 2007), it is obvious to combine prior art elements according to known methods to yield predictable results, as would be the case here. With regard to applicant's argument that there is no teaching to create an environmentally limited viability system, the combination as set forth would create a cell with the same characteristics as currently claimed. It does not matter whether one calls it an "environmentally limited viability system" or a "balanced-lethal" system. The fact remains that applicant has claimed a strain with the *asd* gene deleted from the chromosome and supplied on an extrachromosomal vector, linked to a promoter that would be expressed in a permissive environment (in this case, the presence of arabinose) and which would not be expressed in a non-permissive environment (the absence of arabinose). This is exactly what one would have after combining the Guzman and Galan references. Moreover, there is no requirement that the motivation to combine references set forth by the examiner be the same as the motivation that applicant has used.

As outlined previously, the instant claims are drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the permissive environment comprises an environment containing arabinose to maintain expression of the essential gene and the non-permissive environment comprises an environment lacking arabinose (claim 63); wherein the essential gene comprises the *asd* gene operatively linked to *araC*-Pbad (claim 67); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the

essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment (temperature greater than 30°C or the presence of arabinose) and is not expressed when the cell is in the non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose); wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment (claim 84). Claims are also drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94).

Galan *et al.* disclose an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (see abstract). The *asd* gene is connected to a P_{trc} promoter, which serves as a means of engineered expression (see page 32, column 1).

Galan *et al.* differs from the instant application in that the essential gene is not linked to *araC*-Pbad and the permissive environment is not disclosed as containing a nutrient required to maintain expression of the essential gene.

Guzman *et al.* disclose plasmid vectors containing the *araC*-Pbad promoter (see abstract). In the presence of arabinose, transcription from this promoter is turned on, and in its absence, transcription only occurs at very low levels (page 4121, column 2, paragraph 2). Guzman *et al.*

also teach that the tight control of expression provided by the *araC*-Pbad promoter system is an important characteristic that is mostly absent in other available expression systems, and that this feature has been indispensable in the isolation and study of null mutations in essential genes and in the evaluation of the depletion phenotype of these genes (page 4128, column 2, paragraph 2). Guzman *et al.* further teach that it is useful to express a cloned gene from an inducible promoter and assess the effect of the expression or depletion of the gene product in mutants lacking the chromosomal gene, and that in these situations, it is highly desirable to use a system (such as the *araC*-Pbad system) that can be efficiently shut off (page 4121, column 1, paragraph 1).

Therefore, it would have been obvious to one of skill in the art to use the *araC*-Pbad promoter system (as disclosed by Guzman *et al.*) to control expression of the *asd* gene in the bacterial cells of Galan *et al.* because it is useful to use such a promoter to control expression in the study of depletion phenotypes.

One would have had a reasonable expectation of success because the *araC*-Pbad promoter system (as disclosed by Guzman *et al.*) has been shown to control expression gene expression in bacterial cells (see abstract).

The rejection of claims 61-62, 64, 70-73, 83-86, and 88-94 under 35 U.S.C. 103(a) as being unpatentable over Galan *et al.* (Gene, 94:29-35, 1990) in view of Glick *et al.* (Molecular Biotechnology, Principles and Applications of Recombinant DNA, 1994, ASM Press, pp. 90-92), is maintained for the reasons set forth in the previous office action.

Applicant argues:

1. That Galan does not teach or suggest regulating the expression of *asd*. Instead, Galan teaches the use of "balanced-lethal" technology, where the *asd* gene is deleted from the bacterial chromosome and is supplied on a plasmid, to serve as a selection pressure to maintain the plasmid.

2. That there is no teaching or suggestion in Galan or Glick to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another. Applicant asserts that this system is useful for vaccine microorganisms.

Applicant's arguments have been fully considered and deemed non-persuasive.

Regarding argument 1, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Regarding argument 2, the motivation to combine was set forth in the previous office action. As stated previously, Galan teaches a bacterial strain where the *asd* gene has been deleted from the bacterial chromosome and is supplied on a plasmid. Glick teaches the use of a temperature sensitive promoter (the same promoter disclosed by applicant in the instant specification). Glick further teaches that this promoter is useful in controlling transcription to avoid a high level of continual expression which is often detrimental to a host cell. Therefore, one would have been motivated to link *asd* to the promoter of Glick to control detrimental high-level transcription. Further, according to *KSR International Co. v. Teleflex Inc.*, No. 04-1350 (U.S. Apr. 30, 2007), it is obvious to combine prior art elements according to known methods to yield predictable results, as would be the case here. With regard to applicant's argument that there is no teaching to create an environmentally limited viability system, the combination as set forth would create a cell with the same characteristics as currently claimed. It does not matter whether one calls it an "environmentally limited viability system" or a "balanced-lethal" system. The fact remains that applicant has claimed a strain with the *asd* gene deleted from the chromosome and supplied on an extrachromosomal vector, linked to a promoter that would be expressed in a permissive environment (in this case, the presence of arabinose) and which would not be expressed in a non-permissive environment (the absence of arabinose). This is exactly what one would have after combining the Guzman and Glick references. Moreover, there is no requirement that the motivation to combine references set forth by the examiner be the same as the motivation that applicant has used.

As outlined previously, the instant claims are drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the

cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal (claims 64, 86, 90); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment (temperature greater than 30°C or the presence of arabinose) and is not expressed when the cell is in the non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose); wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment (claim 84); wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C (claims 85 and 89). Claims are also drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94).

Galan *et al.* disclose an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (see abstract). The *asd* gene is connected to a P_{trc} promoter, which serves as a means of engineered expression (see page 32, column 1).

Galan *et al.* differs from the instant application in that Galan *et al.* do not disclose the permissive environment as inside a warm-blooded animal and the non-permissive environment as outside a warm-blooded animal, or the permissive environment as comprising a temperature of about 37°C and the non-permissive environment as comprising a temperature of less than about 30°C.

Glick *et al.* disclose the regulatable strong promoter *p*L, which is controlled by the *c*I repressor protein of bacteriophage λ. A temperature sensitive mutant of this repressor, cI_{857} is used to regulate expression so that transcription can proceed at 42°C, but not at 30°C (page 91, paragraph 2). It is noted that this is the same repressor system disclosed in the instant specification to allow growth at 37°C (the temperature inside a warm-blooded animal), but not at 30°C (a temperature outside a warm-blooded animal). Glick *et al.* further teach that the use of regulatable strong promoters is advantageous, and that it is desirable to control transcription in such a way that a cloned gene is expressed only at a specific stage in the host cell growth cycle and only for a specified duration, because a high level of continual expression of a cloned gene is often detrimental to the host cell (page 90, paragraph 2).

Therefore, it would have been obvious to one of skill in the art to use the temperature-regulated *p*L promoter system (as disclosed by Glick *et al.*) to control expression of the *asd* gene in the bacterial cells of Galan *et al.* because regulatable strong promoters are advantageous to avoid a high level of continual expression of a cloned gene which is often detrimental to the host cell.

One would have had a reasonable expectation of success because the use of the temperature-regulated *p*L promoter system in order to control gene expression (as disclosed by Glick *et al.*) is well known in the art (as evidenced by the fact that Glick *et al.* is a textbook).

The rejection of claims 61-63, 67, 70-73, 83-84, 87-88, and 91-94 are rejected under 35 U.S.C. 103(a) as being unpatentable over Curtiss (US Patent 5,840,483) in view of Guzman *et al.*

(J. Bacteriol., 177:4121-4130, 1995), is maintained for the reasons set forth in the previous office action.

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

Applicant argues:

1. That Curtiss teaches the use of "balanced-lethal" technology, which does not achieve biological containment because the cells survive due to the presence of the essential gene on the plasmid.

2. That there is no teaching or suggestion in Guzman to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another.

Applicant's arguments have been fully considered and deemed non-persuasive.

Regarding arguments 1 and 2, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Regarding argument 2, the motivation to combine was set forth in the previous office action. As stated previously, Curtiss teaches a bacterial strain where the *asd* gene has been deleted from the bacterial chromosome and is supplied on a plasmid. Guzman teaches a promoter system that would allow expression only in the presence of arabinose (the same promoter system used by applicant in claim 67). Guzman further teaches that it is useful to link their promoter system to essential genes to study the depletion phenotype created by a null mutation. Therefore, one would have been motivated to link *asd* to the promoter of Guzman to study depletion phenotypes. Further, according to *KSR International Co. v. Teleflex Inc.*, No. 04-1350 (U.S. Apr. 30, 2007), it is obvious to combine prior art elements according to known methods to yield predictable results, as would be the case here. With regard to applicant's argument that there is no teaching to create an environmentally limited viability system, the

combination as set forth would create a cell with the same characteristics as currently claimed. It does not matter whether one calls it an "environmentally limited viability system" or a "balanced-lethal" system. The fact remains that applicant has claimed a strain with the *asd* gene deleted from the chromosome and supplied on an extrachromosomal vector, linked to a promoter that would be expressed in a permissive environment (in this case, the presence of arabinose) and which would not be expressed in a non-permissive environment (the absence of arabinose). This is exactly what one would have after combining the Guzman and Curtiss references. Moreover, there is no requirement that the motivation to combine references set forth by the examiner be the same as the motivation that applicant has used.

As outlined previously, the instant claims are drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the permissive environment comprises an environment containing arabinose to maintain expression of the essential gene and the non-permissive environment comprises an environment lacking arabinose (claim 63); wherein the essential gene comprises the *asd* gene operatively linked to *araC*-Pbad (claim 67); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment (temperature greater than 30°C or the presence of arabinose) and is not expressed

when the cell is in the non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose); wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment (claim 84). Claims are also drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94).

Curtiss discloses an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (col. 9, line 25- col. 10. line 35; claims 6-9).

Curtiss differs from the instant application in that the essential gene is not linked to *araC*-Pbad and the permissive environment is not disclosed as containing a nutrient required to maintain expression of the essential gene.

Guzman *et al.* disclose plasmid vectors containing the *araC*-Pbad promoter (see abstract). In the presence of arabinose, transcription from this promoter is turned on, and in its absence, transcription only occurs at very low levels (page 4121, column 2, paragraph 2). Guzman *et al.* also teach that the tight control of expression provided by the *araC*-Pbad promoter system is an important characteristic that is mostly absent in other available expression systems, and that this feature has been indispensable in the isolation and study of null mutations in essential genes and in the evaluation of the depletion phenotype of these genes (page 4128, column 2, paragraph 2). Guzman *et al.* further teach that it is useful to express a cloned gene from an inducible promoter and assess the effect of the expression or depletion of the gene product in mutants lacking the

chromosomal gene, and that in these situations, it is highly desirable to use a system (such as the *araC*-Pbad system) that can be efficiently shut off (page 4121, column 1, paragraph 1).

Therefore, it would have been obvious to one of skill in the art to use the *araC*-Pbad promoter system (as disclosed by Guzman *et al.*) to control expression of the *asd* gene in the bacterial cells of Curtiss because it is useful to use such a promoter to control expression in the study of depletion phenotypes.

One would have had a reasonable expectation of success because the *araC*-Pbad promoter system (as disclosed by Guzman *et al.*) has been shown to control expression gene expression in bacterial cells (see abstract).

The rejection of claims 61-63, 67, 70-73, 83-84, 88, and 91-94 under 35 U.S.C. 103(a) as being unpatentable over Curtiss (US Patent 5,672,345) in view of Guzman *et al.* (J. Bacteriol., 177:4121-4130, 1995), is maintained for the reasons set forth in the previous office action.

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

Applicant argues:

1. That Curtiss teaches the use of "balanced-lethal" technology, which does not achieve biological containment because the cells survive due to the presence of the essential gene on the plasmid.

2. That there is no teaching or suggestion in Guzman to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another.

Applicant's arguments have been fully considered and deemed non-persuasive.

Regarding arguments 1 and 2, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Regarding argument 2, the motivation to combine was set forth in the previous office action. As stated previously, Curtiss teaches a bacterial strain where the *asd* gene has been deleted from the bacterial chromosome and is supplied on a plasmid. Guzman teaches a promoter system that would allow expression only in the presence of arabinose (the same promoter system used by applicant in claim 67). Guzman further teaches that it is useful to link their promoter system to essential genes to study the depletion phenotype created by a null mutation. Therefore, one would have been motivated to link *asd* to the promoter of Guzman to study depletion phenotypes. Further, according to *KSR International Co. v. Teleflex Inc.*, No. 04-1350 (U.S. Apr. 30, 2007), it is obvious to combine prior art elements according to known methods to yield predictable results, as would be the case here. With regard to applicant's argument that there is no teaching to create an environmentally limited viability system, the combination as set forth would create a cell with the same characteristics as currently claimed. It does not matter whether one calls it an "environmentally limited viability system" or a "balanced-lethal" system. The fact remains that applicant has claimed a strain with the *asd* gene deleted from the chromosome and supplied on an extrachromosomal vector, linked to a promoter that would be expressed in a permissive environment (in this case, the presence of arabinose) and which would not be expressed in a non-permissive environment (the absence of arabinose). This is exactly what one would have after combining the Guzman and Curtiss references. Moreover, there is no requirement that the motivation to combine references set forth by the examiner be the same as the motivation that applicant has used.

As outlined previously, the instant claims are drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the permissive environment comprises an environment containing arabinose to maintain expression of the

essential gene and the non-permissive environment comprises an environment lacking arabinose (claim 63); wherein the essential gene comprises the *asd* gene operatively linked to *araC*-Pbad (claim 67); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment (temperature greater than 30°C or the presence of arabinose) and is not expressed when the cell is in the non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose); wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment (claim 84). Claims are also drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94).

Curtiss discloses an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (col. 9, line 25- col. 10. line 35; claims 6-9).

Curtiss differs from the instant application in that the essential gene is not linked to *araC*-Pbad and the permissive environment is not disclosed as containing a nutrient required to maintain expression of the essential gene.

Guzman *et al.* disclose plasmid vectors containing the *araC*-Pbad promoter (see abstract). In the presence of arabinose, transcription from this promoter is turned on, and in its absence, transcription only occurs at very low levels (page 4121, column 2, paragraph 2). Guzman *et al.* also teach that the tight control of expression provided by the *araC*-Pbad promoter system is an important characteristic that is mostly absent in other available expression systems, and that this feature has been indispensable in the isolation and study of null mutations in essential genes and in the evaluation of the depletion phenotype of these genes (page 4128, column 2, paragraph 2). Guzman *et al.* further teach that it is useful to express a cloned gene from an inducible promoter and assess the effect of the expression or depletion of the gene product in mutants lacking the chromosomal gene, and that in these situations, it is highly desirable to use a system (such as the *araC*-Pbad system) that can be efficiently shut off (page 4121, column 1, paragraph 1).

Therefore, it would have been obvious to one of skill in the art to use the *araC*-Pbad promoter system (as disclosed by Guzman *et al.*) to control expression of the *asd* gene in the bacterial cells of Curtiss because it is useful to use such a promoter to control expression in the study of depletion phenotypes.

One would have had a reasonable expectation of success because the *araC*-Pbad promoter system (as disclosed by Guzman *et al.*) has been shown to control expression gene expression in bacterial cells (see abstract).

The rejection of claims 61-62, 64, 70-73, 83-86, and 88-94 under 35 U.S.C. 103(a) as being unpatentable over Curtiss (US Patent 5,840,483) in view of Glick *et al.* (Molecular Biotechnology, Principles and Applications of Recombinant DNA, 1994, ASM Press, pp. 90-92), is maintained for the reasons set forth in the previous office action.

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the

inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

Applicant argues:

1. That Curtiss teaches the use of "balanced-lethal" technology, which does not achieve biological containment because the cells survive due to the presence of the essential gene on the plasmid.

2. That there is no teaching or suggestion in Glick to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another.

Applicant's arguments have been fully considered and deemed non-persuasive.

Regarding arguments 1 and 2, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Regarding argument 2, the motivation to combine was set forth in the previous office action. As stated previously, Curtiss teaches a bacterial strain where the *asd* gene has been deleted from the bacterial chromosome and is supplied on a plasmid. Glick teaches the use of a temperature sensitive promoter (the same promoter disclosed by applicant in the instant specification). Glick further teaches that this promoter is useful in controlling transcription to avoid a high level of continual expression which is often detrimental to a host cell. Therefore, one would have been motivated to link *asd* to the promoter of Glick to control detrimental high-level transcription. Further, according to *KSR International Co. v. Teleflex Inc.*, No. 04-1350 (U.S. Apr. 30, 2007), it is obvious to combine prior art elements according to known methods to yield predictable results, as would be the case here. With regard to applicant's argument that there is no teaching to create an environmentally limited viability system, the combination as set forth would create a cell with the same characteristics as currently claimed. It does not matter whether one calls it an "environmentally limited viability system" or a "balanced-lethal" system. The fact remains that applicant has claimed a strain with the *asd* gene deleted from the chromosome and supplied on an extrachromosomal vector, linked to a promoter that would be expressed in a permissive environment (in this case, the presence of arabinose) and which would not be expressed in a non-permissive environment (the absence of arabinose). This is exactly

what one would have after combining the Curtiss and Glick references. Moreover, there is no requirement that the motivation to combine references set forth by the examiner be the same as the motivation that applicant has used.

As outlined previously, the instant claims are drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal (claims 64, 86, 90); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment (temperature greater than 30°C or the presence of arabinose) and is not expressed when the cell is in the non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose); wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment (claim 84); wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C (claims 85 and 89). Claims are also drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or

equal to 30°C or the presence of arabinose), the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94).

Curtiss discloses an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (col. 9, line 25- col. 10. line 35; claims 6-9).

Curtiss differs from the instant application in that Curtiss does not disclose the permissive environment as inside a warm-blooded animal and the non-permissive environment as outside a warm-blooded animal, or the permissive environment as comprising a temperature of about 37°C and the non-permissive environment as comprising a temperature of less than about 30°C.

Glick *et al.* (Molecular Biotechnology, Principles and Applications of Recombinant DNA, 1994, ASM Press, pp. 90-92) disclose the regulatable strong promoter *p*L, which is controlled by the *c*I repressor protein of bacteriophage λ. A temperature sensitive mutant of this repressor, cI_{857} is used to regulate expression so that transcription can proceed at 42°C, but not at 30°C (page 91, paragraph 2). It is noted that this is the same repressor system disclosed in the instant specification to allow growth at 37°C (the temperature inside a warm-blooded animal), but not at 30°C (a temperature outside a warm-blooded animal). Glick *et al.* further teach that the use of regulatable strong promoters is advantageous, and that it is desirable to control transcription in such a way that a cloned gene is expressed only at a specific stage in the host cell growth cycle and only for a specified duration, because a high level of continual expression of a cloned gene is often detrimental to the host cell (page 90, paragraph 2).

Therefore, it would have been obvious to one of skill in the art to use the temperature-regulated *p*L promoter system (as disclosed by Glick *et al.*) to control expression of the *asd* gene in the bacterial cells of Curtiss because regulatable strong promoters are advantageous to avoid a high level of continual expression of a cloned gene which is often detrimental to the host cell.

One would have had a reasonable expectation of success because the use of the temperature-regulated *p*L promoter system in order to control gene expression (as disclosed by Glick *et al.*) is well known in the art (as evidenced by the fact that Glick *et al.* is a textbook).

The rejection of claims 61-62, 64, 70-73, 83-86, and 88-94 under 35 U.S.C. 103(a) as being unpatentable over Curtiss (US Patent 5,672,345) in view of Glick *et al.* (Molecular Biotechnology, Principles and Applications of Recombinant DNA, 1994, ASM Press, pp. 90-92), is maintained for the reasons set forth in the previous office action.

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

Applicant argues:

1. That Curtiss teaches the use of "balanced-lethal" technology, which does not achieve biological containment because the cells survive due to the presence of the essential gene on the plasmid.

2. That there is no teaching or suggestion in Glick to create an environmentally limited viability system wherein a cell is viable in one environment and not viable in another.

Applicant's arguments have been fully considered and deemed non-persuasive.

Regarding arguments 1 and 2, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Regarding argument 2, the motivation to combine was set forth in the previous office action. As stated previously, Curtiss teaches a bacterial strain where the *asd* gene has been deleted from the bacterial chromosome and is supplied on a plasmid. Glick teaches the use of a temperature sensitive promoter (the same promoter disclosed by applicant in the instant specification). Glick further teaches that this promoter is useful in controlling transcription to

avoid a high level of continual expression which is often detrimental to a host cell. Therefore, one would have been motivated to link *asd* to the promoter of Glick to control detrimental high-level transcription. Further, according to *KSR International Co. v. Teleflex Inc.*, No. 04-1350 (U.S. Apr. 30, 2007), it is obvious to combine prior art elements according to known methods to yield predictable results, as would be the case here. With regard to applicant's argument that there is no teaching to create an environmentally limited viability system, the combination as set forth would create a cell with the same characteristics as currently claimed. It does not matter whether one calls it an "environmentally limited viability system" or a "balanced-lethal" system. The fact remains that applicant has claimed a strain with the *asd* gene deleted from the chromosome and supplied on an extrachromosomal vector, linked to a promoter that would be expressed in a permissive environment (in this case, the presence of arabinose) and which would not be expressed in a non-permissive environment (the absence of arabinose). This is exactly what one would have after combining the Curtiss and Glick references. Moreover, there is no requirement that the motivation to combine references set forth by the examiner be the same as the motivation that applicant has used.

As outlined previously, the instant claims are drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal (claims 64, 86, 90); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to

a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment (temperature greater than 30°C or the presence of arabinose) and is not expressed when the cell is in the non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose); wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment (claim 84); wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C (claims 85 and 89). Claims are also drawn to an isolated bacterial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment (temperature greater than 30°C or the presence of arabinose) and non-viable in a non-permissive environment (temperature less than or equal to 30°C or the presence of arabinose), the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94).

Curtiss discloses an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (col. 8, line 66- col. 10. line 11; claims 1, 3, and 7-8). The instant specification defines a permissive environment as an environment in which the claimed cells are viable, and a non-permissive environment as one in which the cells are non-viable or temporarily viable. As all gene expression would cease when a cell dies (becomes non-viable), the essential gene (*asd*) of the cells disclosed by Curtiss would not be expressed in a non-permissive environment.

Curtiss differs from the instant application in that Curtiss does not disclose the permissive environment as inside a warm-blooded animal and the non-permissive environment as outside a

warm-blooded animal, or the permissive environment as comprising a temperature of about 37°C and the non-permissive environment as comprising a temperature of less than about 30°C.

Glick *et al.* (Molecular Biotechnology, Principles and Applications of Recombinant DNA, 1994, ASM Press, pp. 90-92) disclose the regulatable strong promoter *p*L, which is controlled by the *c*I repressor protein of bacteriophage λ. A temperature sensitive mutant of this repressor, cI_{857} is used to regulate expression so that transcription can proceed at 42°C, but not at 30°C (page 91, paragraph 2). It is noted that this is the same repressor system disclosed in the instant specification to allow growth at 37°C (the temperature inside a warm-blooded animal), but not at 30°C (a temperature outside a warm-blooded animal). Glick *et al.* further teach that the use of regulatable strong promoters is advantageous, and that it is desirable to control transcription in such a way that a cloned gene is expressed only at a specific stage in the host cell growth cycle and only for a specified duration, because a high level of continual expression of a cloned gene is often detrimental to the host cell (page 90, paragraph 2).

Therefore, it would have been obvious to one of skill in the art to use the temperature-regulated *p*L promoter system (as disclosed by Glick *et al.*) to control expression of the *asd* gene in the bacterial cells of Curtiss because regulatable strong promoters are advantageous to avoid a high level of continual expression of a cloned gene which is often detrimental to the host cell. One would have had a reasonable expectation of success because the use of the temperature-regulated *p*L promoter system in order to control gene expression (as disclosed by Glick *et al.*) is well known in the art (as evidenced by the fact that Glick *et al.* is a textbook).

Conclusion

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37

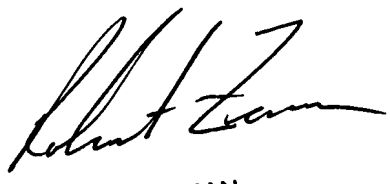
CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Brian J. Gangle whose telephone number is (571) 272-1181. The examiner can normally be reached on M-F 8-4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's acting supervisor, Bruce Campell can be reached on (571) 272-0974. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Brian Gangle
AU 1645



ROBERT A. ZEMAN
PRIMARY EXAMINER